



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

January 9, 2015

BIO-RAD LABORATORIES
JUANG WANG
REGULATORY AFFAIRS REPRESENTATIVE
5500 E. 2ND STREET
BENICIA CA 94510

Re: K141114

Trade/Device Name: BioPlex® 2200 25-OH Vitamin D Kit
BioPlex® 2200 25-OH Vitamin D Calibrator Set,
BioPlex® 2200 25-OH Vitamin D Control Set

Regulation Number: 21 CFR 862.1825

Regulation Name: Vitamin D test system

Regulatory Class: II

Product Code: MRG, JJJ, JIT

Dated: November 25, 2014

Received: November 25, 2014

Dear Juang Wang:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>. Also, please note the regulation entitled, “Misbranding by reference to premarket notification” (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH’s Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address

<http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>.

Sincerely yours,

Stayce Beck -S

For :Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (*if known*)

K141114

Device Name

BioPlex® 2200 25-OH Vitamin D Kit
BioPlex® 2200 25-OH Vitamin D Calibrator Set
BioPlex® 2200 25-OH Vitamin D Control Set

Indications for Use (*Describe*)

The BioPlex® 2200 25-OH Vitamin D kit is a flow competitive immunoassay intended for the quantitative determination of 25-hydroxyvitamin D in human serum. The BioPlex 2200 25-OH Vitamin D assay is to be used as an aid in the assessment of vitamin D sufficiency.

The BioPlex® 2200 25-OH Vitamin D kit is intended for use with the Bio-Rad BioPlex 2200 System.

The BioPlex® 2200 25-OH Vitamin D Calibrator Set is intended for the calibration of the BioPlex® 2200 25-OH Vitamin D Reagent Pack.

The BioPlex® 2200 25-OH Vitamin D Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex® 2200 System and the corresponding BioPlex® 25-OH Vitamin D Reagent Packs in the clinical laboratory. The performance of the BioPlex® 25-OH Vitamin D Control Set has not been established with any other 25-hydroxyvitamin D assays.

Type of Use (*Select one or both, as applicable*)

Prescription Use (Part 21 CFR 801 Subpart D)

Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

BioPlex® 2200 25-OH Vitamin D 510(k) Summary

Bio-Rad Laboratories hereby submits this 510(k) in accordance with the requirements of SMDA 1990 and 21 CFR 807.92. This summary of 510(k) safety and effectiveness information provides detail as a basis for a determination of substantial equivalence for the BioPlex® 2200 25-OH Vitamin D kit.

510(k) Number:

k141114

Summary Preparation Date:

December 30, 2014

Applicant:

Bio-Rad Laboratories

Contact:

Juang Wang

Regulatory Affairs Representative

5500 E. Second Street

Benicia, CA 94510

Tel: 510-741-4609

FAX: 510-741-3941

Juang_wang@bio-rad.com

Purpose for Submission:

New Device

Measurand:

25-hydroxyvitamin D

Type of Test:

Quantitative multiplexed flow immunoassay

Proprietary and Established Names:

BioPlex® 2200 25-OH Vitamin D kit

BioPlex® 2200 25-OH Vitamin D Calibrator Set

BioPlex® 2200 25-OH Vitamin D Control Set

Regulatory Information:

1. Regulation section:

21 CFR §862.1825 – Vitamin D test system

21 CFR §862.1150 – Calibrator

21 CFR §862.1660 – Quality Control Material (assayed and unassayed)

2. Classification:

Class II (Assays, Calibrator)
Class I (Controls)

3. Product code:

MRG, System, Test, Vitamin D
JIT, Calibrator, Secondary
JJX, Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

Clinical Chemistry (75)

Intended Use:

1. Intended use(s):

The BioPlex® 2200 25-OH Vitamin D kit is a flow competitive immunoassay intended for the quantitative determination of 25-hydroxyvitamin D in human serum. The BioPlex 2200 25-OH Vitamin D assay is to be used as an aid in the assessment of vitamin D sufficiency.

The BioPlex® 2200 25-OH Vitamin D kit is intended for use with the Bio-Rad BioPlex 2200 System.

The BioPlex® 2200 25-OH Vitamin D Calibrator Set is intended for the calibration of the BioPlex® 2200 25-OH Vitamin D Reagent Pack.

The BioPlex® 2200 25-OH Vitamin D Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex® 2200 System and the corresponding BioPlex® 25-OH Vitamin D Reagent Packs in the clinical laboratory. The performance of the BioPlex® 25-OH Vitamin D Control Set has not been established with any other 25-hydroxyvitamin D assays.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Bio-Rad BioPlex® 2200 System

Device Description:

BioPlex® 2200 25-OH Vitamin D kit includes the following components:

- One (1) 10 mL vial of Bead Set containing dyed beads coated with anti-25-OH D antibody (sheep), an Internal Standard bead (ISB), and a Serum Verification bead

(SVB) in buffer with protein stabilizers (bovine). ProClin 950 (< 1.0%) and sodium azide (< 0.1%) as preservatives.

- One (1) 10 mL vial of Release Buffer containing 25-OH Vitamin D releasing reagents in citrate and trisodium citrate acid buffer at pH 4.1 and ProClin 950 (<1.0%) as preservative.
- One (1) 5 mL vial of Conjugate 1 containing biotinylated 25-OH Vitamin D conjugate and biotinylated anti-human FXIII antibody conjugate (murine) in buffer with protein stabilizers (bovine). ProClin 950 (< 1.0%) and 5-bromo-5-nitro-1, 3-dioxane (<0.1%) as preservatives and chemical blockers.
- One (1) 5 mL vial of Conjugate 2 containing phycoerythrin conjugated streptavidin (SA -PE) in a buffer comprising protein stabilizers (bovine). ProClin 950 (< 1.0%) and sodium azide (< 0.1%) as preservatives, chemical blockers and detergent (Tween 20).

BioPlex® 2200 25-OH Vitamin D Calibrator set contains six (6) 0.5 mL 25-OH Vitamin D vials. Calibrator level 1 contains 25% horse serum without 25-OH Vitamin D. The calibrator levels 2 to 6 are provided in a Vitamin D depleted human serum matrix supplemented with known concentration of 25-hydroxyvitamin D₃. All calibrators contain ProClin 950 (\leq 0.3%), sodium benzoate (\leq 0.1%) and 5-bromo-5-nitro-1, 3-dioxane (<0.1%) as preservatives.

BioPlex® 2200 25-OH Control set contains two (2) 1.5 mL Level 1 and two (2) 1.5 mL Level 2 Control vials, each containing 25-OH Vitamin D in human serum matrix. All controls contain ProClin 950 (\leq 0.3%), sodium benzoate (\leq 0.1%) and 5-bromo-5-nitro-1, 3-dioxane (<0.1%) as preservatives.

Additional materials required but not supplied include BioPlex® 2200 Sheath Fluid containing Phosphate Buffered Saline (PBS), ProClin 300 (0.03%) and sodium azide (<0.1%) as preservatives; and BioPlex® 2200 Wash Solution containing Phosphate Buffered Saline (PBS) and Tween 20. ProClin 300 (0.03%) and sodium azide (<0.1%) as preservatives.

Substantial Equivalence Information:

1. Predicate device name(s):
EUROIMMUN 25-OH Vitamin D ELISA, k123660
2. Comparison with predicate:

Device Similarities		
Characteristics	New Device BioPlex 2200 25-OH Vitamin D Kit	Predicate Device EUROIMMUN 25-OH Vitamin D ELISA K123660
Intended Use/Indication for Use	Intended for the quantitative determination of 25-hydroxyvitamin D in serum. To be used as an aid in the assessment of vitamin D sufficiency	Same
Measured Analyte	25-hydroxyvitamin D	Same
Assay Type	Quantitative	Same
Test Principle	Competitive immunoassay	Same
Antibody	Monoclonal Sheep antibody against 25-OH Vitamin D	Same
Signal Detection	Fluorescence	Same
Unit of Measure	ng/mL	Same
Calibrator(s) and Calibration	6 calibrator levels (sold separately); 4-PL (parameter logistic) curve fit algorithm	Same

Device Differences		
Characteristics	New Device BioPlex 2200 25-OH Vitamin D Kit	Predicate Device EUROIMMUN 25-OH Vitamin D ELISA K123660
Assay Technology	Automated flow competitive immunoassay	Manual competitive immunoassay
Conjugate	Biotinylated 25-hydroxyvitamin D and phycoerythrin conjugated streptavidin	Biotin-labeled 25-OH vitamin D, Peroxidase-labeled streptavidin and substrate TMB
Solid Phase	Antibody-coated paramagnetic microbeads	Antibody coated 96 microwell ELISA plate
Measuring range	6.5 – 125.0 ng/mL	4 – 120 ng/mL
Sample Matrix	Serum	Serum or EDTA or Lithium heparin plasma
Sample Size	10 µL	20µL
Calibrator Matrix	25% horse serum and depleted human serum	Liquid in horse serum with preservatives

Device Differences		
Characteristics	New Device BioPlex 2200 25-OH Vitamin D Kit	Predicate Device EUROIMMUN 25-OH Vitamin D ELISA K123660
	with ProClin 950, sodium benzoate and BND	
Open Pack Stability	60 days	Not applicable
Reagent Integral Storage	On-board or in refrigerator at 2-8°C	Not applicable
Sample Handling/Process	Automated	Manual
Calibrator Open storage at 2-8°C	30 days	3 months
Calibration Frequency	Every 30 days	Every 96 well plate
Instrumentation	Bio-Rad BioPlex 2200 System	ELISA plate reader
Measuring wavelength	550 – 610 nm	450/620 nm

Control Set Similarities and Differences		
Characteristics	BioPlex 2200 25-OH Vitamin D Kit	Predicate Device EUROIMMUN 25-OH Vitamin D ELISA K123660
Intended Use	Use as an assayed quality control to monitor the overall performance of 25-OH Vitamin D reagent.	Same
Storage	Store at 2 -8°C until ready to use	Same
Matrix	Human serum with ProClin 950, sodium benzoate and BND	Liquid in horse serum with preservatives
Control Open Stability at 2 – 8°C	60 days	No Applicable

Standard/Guidance Document Referenced (if applicable):

EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition (Vol. 24 No.25)

EP06-A, Evaluation of Linearity of Quantitative Measurement: A Statistical Approach, Approved Guideline (Vol. 23 No.16)

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition (Vol. 25 No.27)
EP09-A2IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision) (Vol. 30 No. 17)
EP15-A2, User Verification of Performance for Precision and Trueness, Approved Guideline, Second Edition (Vol. 25 No.17)
EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition (Vol. 32 No.8)
EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline (Vol. 29, No. 20)
C28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition (Vol. 28 No.30)

Test Principle:

The BioPlex® 2200 25-OH Vitamin D assay is a multiplesflow competitive immunoassay for the quantitative determination of 25-hydroxyvitamin D in human serum.

The BioPlex 2200 System combines an aliquot of patient sample with the Vitamin D Release Buffer to dissociate the 25-hydroxyvitamin D from its binding protein. After the first incubation, the bead reagent is added to the reaction vessel and incubated at 37°C. After the second incubation, the BioPlex 2200 System adds the vitamin D-biotin conjugate 1. The excess conjugate 1 is removed during a wash cycle and the streptavidin-phcoerythrin (SA-PE) conjugate 2 is added. The excess conjugate 2 is removed during a wash cycle, and the beads are re-suspended in wash buffer. The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the dyes, and the amount of 25-hydroxyvitamin D captured is inversely proportional to the fluorescence of the attached SA-PE. Raw data is calculated in relative fluorescence intensity (RFI). Two additional dyed beads, Internal Standard Bead (ISB) and Serum Verification Bead (SVB) are present in each reaction mixture to verify detector response and the addition of serum to the reaction vessel, respectively.

The BioPlex 25-OH Vitamin D assay is calibrated using a set of 6 distinct calibrators supplied separately by Bio-Rad Laboratories. Results are calculated from a 4-PL (parameter logistic) calibration curve in which an inverse relationship exists between the amount of 25-hydroxyvitamin D in the patient sample and the amount of RFI detected by the system. The results are expressed in ng/mL.

Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*
Precision testing of the BioPlex® 2200 25-OH Vitamin D kit on the BioPlex® 2200 instrument was performed in accordance with CLSI EP5-A2 guideline. A human serum panel consisting of 6 frozen samples spanning the measuring range was assayed in duplicate per run on two runs daily over 20 days (N=80) on one

reagent lot. Two levels of the BioPlex 25-OH Vitamin D controls were also included. The data were analyzed for within-run, between-run, between-day, and total precision and the mean (ng/mL), standard deviation (ng/mL) and percent coefficient of variation (%CV) are summarized below:

BioPlex® 2200 25-OH Vitamin D – CLSI EP5-A2 Precision

Serum Panel	N	Mean ng/mL	Within Run		Between Run		Between Day		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	15.0	1.19	7.9	0.69	4.60	0.77	5.2	1.58	10.5
Sample 2	80	17.2	1.02	5.9	0.86	5.00	1.19	6.9	1.78	10.4
Sample 3	80	36.1	1.61	4.5	1.30	3.60	1.56	4.3	2.59	7.2
Sample 4	80	47.2	2.20	4.7	1.24	2.60	1.85	3.9	3.13	6.6
Sample 5	80	77.9	1.99	2.6	1.42	1.80	1.98	2.5	3.15	4.0
Sample 6	80	110.8	3.60	3.2	3.00	2.7	2.62	2.4	5.37	4.8
Control 1	80	22.1	1.30	5.9	0.87	3.9	1.04	4.7	1.88	8.5
Control 2	80	50.0	2.38	4.8	1.18	2.4	1.52	3.0	3.07	6.1

CLSI EP15-A2 Reproducibility

Precision and reproducibility was also evaluated in accordance with CLSI EP15-A2 guideline “User Verification of Performance for Precision and Trueness, Vol 25, No 17”.

A different serum panel consisting of 8 samples spanning the measuring range were assayed in 2 replicates per run, two runs per day over 5 days (n=20) using one lot of BioPlex 25-OH Vitamin D kit. Two levels of controls were also included. The data were analyzed for within-run, between run, between day, and total precision and the mean ng/mL, standard deviation and percent coefficient of variation (%CV) are summarized below:

BioPlex® 2200 25-OH Vitamin D - CLSI EP15-A2 Reproducibility

Serum Panel	N	Mean ng/mL	Within Run		Between Run		Total Precision	
			SD	%CV	SD	%CV	SD	%CV
Sample 1	20	11.5	0.69	6.0%	0.47	4.1%	1.71	14.8%
Sample 2	20	13.6	0.80	5.9%	0.26	1.9%	1.18	8.7%
Sample 3	20	26.1	0.88	3.4%	1.15	4.4%	1.59	6.1%
Sample 4	20	30.2	1.99	6.6%	0.00	0.0%	2.71	9.0%
Sample 5	20	50.2	2.23	4.4%	0.77	1.5%	2.96	5.9%
Sample 6	20	56.4	2.09	3.7%	2.79	4.9%	5.26	9.3%
Sample 7	20	100.5	4.52	4.5%	2.81	2.8%	5.32	5.3%
Sample 8	20	104.9	3.97	3.8%	1.54	1.5%	5.24	5.0%
Control 1	20	21.6	0.98	4.5%	1.00	4.6%	1.84	8.5%
Control 2	20	58.8	2.44	4.2%	1.29	2.2%	2.99	5.1%

b. Linearity/assay reportable range:

Five high patient serum samples extending 20% higher than upper limit of the assay range were tested to demonstrate linearity. These samples were serially diluted with low levels of human sample near LoQ in accordance with CLSI EP06-A guideline. Each sample and dilution was evaluated in replicates of four using one BioPlex 25-OH Vitamin D reagent lot on one instrument. Linear and polynomial regression analysis of 25-OH Vitamin D recovery *vs.* sample dilution was performed to determine if the dilution curves exhibit statistically significant non-linear regression based on the CLSI guideline EP06-A.

See one example below for the regression parameters (slope, intercept and r^2) of the observed values *vs.* predicted values.

Conc (ng/mL)	Slope	Intercept	r^2	Dilution range
168.9	1.0001	0.0045	0.9988	5.5 – 168.9

The BioPlex 2200 25-OH Vitamin D assay has demonstrated that the assay range is 6.5 to 125.0 ng/mL.

Over-Range (OR) results may be generated for values greater than the reportable measuring range and results are reported as > 125.0 ng/mL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:

The BioPlex 25-OH Vitamin D Calibrators are traceable to internal standards which are determined by UV spectrophotometric analysis using the extinction coefficient of 18,000 mL/mmol/cm at 264 nm wavelength.

The six levels of Master calibrators are manufactured volumetrically from the internal standards into the depleted human serum except level 1 (25-hydroxyvitamin D free) in 25% horse serum. The Master calibrators are immediately frozen at <-70°C.

Value Assignment:

The BioPlex 25-OH Vitamin D kit calibrators are manufactured for each lot kit with the same matrix as the Master calibrators and are stabilized with $\leq 0.3\%$ ProClin 950, $\leq 0.1\%$ sodium benzoate, and $<0.1\%$ 5-bromo-1,3-nitro-dioxane.

Calibrator assignment is established for the matched lot of BioPlex® 2200 25-OH Vitamin D kit using the Master calibrators as reference. Calibrator assignment is established for the matched lot of BioPlex® 2200 25-OH Vitamin D kit using the Master calibrators as reference. For each calibrator level except level 1, three vials are tested in replicates of five on three BioPlex 2200 analyzers for a total of 45 data points. The mean values obtained for each kit calibrator level are verified and must fall within specified acceptable range.

Two levels of the BioPlex 2200 25-OH Vitamin D control set are prepared from a pool of native human serum specimens. For each control level, three vials are tested in replicates of five using each of the kit lots on three BioPlex 2200 analyzers for a total of 45 replicates per reagent lot. The total number of replicates for each control level is 90 when two reagent lots are used and 135 when three reagent lots are used. For each control level, the mean values were derived from replicate analyses and should fall within the corresponding deviation.

The manufacturing target values of the Calibrator and Control Sets are listed below.

Calibrator Set	Target (ng/mL)
Level 1	0.0
Level 2	10.0
Level 3	30.0
Level 4	75.0
Level 5	110.0
Level 6	165.0

Control Set	Target (ng/mL)	Range (ng/mL)
Level 1	19.0	14.5 – 23.5
Level 2	55.0	45.0 – 65.0

Stability:

Stability studies have been performed to support the following claims:

Calibrator and Control:

BioPlex® 2200 25-OH Control and Calibrator Sets: Calibrator Open Vial Stability (2 to 8°C), 30 days from first opening; Control Open Vial Stability (2 to 8°C), 60 days from first opening; Onboard Calibration Curve Stability, 30 days; Calibrators and Controls Real Time Stability (2 to 8°C), 24 months; labeled as until expiration date; Calibrators and Controls Accelerated Stability (2 to 8°C), 2 years predicted. Calibrators freeze-thaw (-20°C or -70°C), 5-freeze thaw cycles; Control freeze-thaw (-20°C or -70°C), 1-freezethaw cycle at -20°C and 5-freeze-thaw cycles at -70°C.

Kit Stability:

BioPlex® 2200 25-OH Vitamin D Kit: Real Time (unopened) Kit Stability, 9 months or until the date of expiration when stored unopened on the instrument or at 2 to 8°C; the open kit claim is 60 days.

Sample Stability:

Sample stability studies were also performed: Sample stability fresh (2 to 8°C), 7 days; Sample stability frozen (-20 or -70°C), 24 months; Sample Freeze-thaw (-20 or -70°C), up to 3-freeze thaw cycles at -20°C and 2-freeze thaw cycles at -

70°C acceptable.

d. Detection limit:

The study was conducted in accordance with CLSI EP17-A2 guideline for determining the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ).

Limit of Blank (LoB)

Five blank samples were tested with two BioPlex 25-OH Vitamin D reagent lots on one instrument in 4 replicates per day for 5 days yielding 100 data points per reagent lot.

A non-parametric statistical analysis at 95th percentile is used to calculate LoB.

Limit of Detection (LoD)

Six human samples with low level of 25-OH vitamin D in the range of 4 to 35 ng/mL were tested with two BioPlex 25-OH Vitamin D reagent lots on one instrument in 12 replicates per day for five days yielding 60 data points per sample per reagent lot. LoD is then calculated by the equation:

$LoD = LoB + c_p SD_{LoD}$ Where c_p is a multiplier to give the 95th percentile of a normal distribution and SD is from the linear regression of standard deviation versus 25-OH Vitamin D mean value.

Limit of Quantitation (LoQ)

The LoQ was evaluated based on the accuracy goal which was defined as precision $\leq 20\%$ CV. The %CV was calculated using the same measurement results of the 6 low level samples used for determining the LoD.

The results of LoB, LoD, and LoQ in ng/mL are summarized in the table below.

LoB	LoD	LoQ
0.8	2.5	6.5

e. Analytical specificity:

Interfering Substances:

An interfering substances study was conducted to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex® 2200 25-OH Vitamin D kit according to CLSI EP7-A2 guideline.

The effects of the test levels of potential interfering substances on the assay have been evaluated with samples containing 10 to 90 ng/mL Vitamin D. The percent difference between the mean value of each test substance and a corresponding control was calculated. No interference was observed with any of the substances

tested if the percent difference is $\leq 10\%$. The substances and the maximum levels of interfering substances tested are shown in the table below:

Substance	Concentration
Hemoglobin*	$\leq 150 \text{ mg/dL}$
Bilirubin (unconjugated)	$\leq 20 \text{ mg/dL}$
Bilirubin (conjugated)	$\leq 30 \text{ mg/dL}$
Triglycerides	$\leq 400 \text{ mg/dL}$
Total Protein	$\leq 12 \text{ g/dL}$
Cholesterol	$\leq 500 \text{ mg/dL}$
Uric Acid	$\leq 20 \text{ mg/dL}$
HAMA	$\leq 100 \text{ ng/mL}$
Rheumatoid Factor	$\leq 350 \text{ IU/mL}$
Ascorbic Acid	$\leq 3 \text{ mg/dL}$

*Hemoglobin $> 150 \text{ mg/dL}$ may interfere. Do not use visibly hemolyzed samples.

Cross-Reactivity:

The study was conducted in accordance with CLSI EP17-A2 using 2 human serum pools at 25-hydroxyvitamin D concentrations of 20 and 35 ng/mL. Nine cross reactants at levels listed below were then spiked into the human serum pools. The spiked and non-spiked samples were then evaluated in replicates of five to calculate the cross reactivity as shown below.

% Cross Reactivity = (spiked vitamin D – non-spiked vitamin D) \div Cross reactant concentration $\times 100\%$

The results of each potential cross reactant are listed below.

Cross Reactant	Spiked Concentration (ng/mL)	% Cross Reactivity
25-hydroxyvitamin D2	30	103%
25-hydroxyvitamin D3	30	97%
Vitamin D2	1000	0.2%
Vitamin D3	1000	0.0%
1,25-dihydroxyvitamin D2	30	>100%
1,25-dihydroxyvitamin D3	30	79%
3-epi 25-hydroxyvitamin D3	30	59%
24,25-dihydroxyvitamin D3	20	9%
Paricalcitol (Zemplar)*	24	>100%

* Paricalcitol (Zemplar) has been found to cross-react and interfere with the BioPlex 2200 25-OH Vitamin D assay

High dose hook effect:

Not Applicable

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparison studies were performed following CLSI EP09-A2-IR guideline.

A total of 204 human samples spanning the entire measuring assay range were tested in singlicate on both the BioPlex 2200 25-OH Vitamin D kit and the predicate assay. Of the 204 samples, there were 185 unaltered samples and 19 samples spiked with 25-hydroxyvitamin D₃ to supplement the assay range. There are eight (8) samples with values lower or higher than the measuring range of the comparator method not including in the analysis. A total of 196 BioPlex 25-OH Vitamin D results were plotted using weighted Deming regression analysis for all samples spanning the measuring range of both assays. Results of the regression slope, intercept, and coefficient of correlation (r) are summarized in the table below:

Number of Results Analyzed	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r) (95%CI)	Test Range (ng/mL)
196	1.0039 (0.9365 to 1.0712)	-0.2256 (-2.4121 to 1.9608)	0.9553 (0.9412 to 0.9661)	BioPlex: 6.6 to 124.9 Comparator: 4.3 to 118.1

b. *Matrix comparison:*

Serum only

3. Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical Specificity:*

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The Expected Values study was conducted following CLSI C28-A3c guideline.

Two hundred and eighty-seven (287) samples from apparently healthy donors including 160 males ranging in age from 21 to 79 and 127 females ranging in age from 21 to 66 were collected from three regions (North, Central, and South) in the US in spring, summer and winter, including African Americans, Hispanics and Caucasians.

The 287 samples from apparently healthy donors met the following inclusion/exclusion criteria as follows and tested with the BioPlex 25-OH Vitamin D kit in singlicate.

- Age from 21 to 90
- Roughly 50% female and 50% male
- 20% from Northern, 20% from Central and 60% from Southern region
- 40% collected in Spring, 30% in Summer and 30% in Winter
- At least 30% African Americans and 30% Caucasians
- 90% not taking Vitamin D supplements and <30% of those taking Vitamin D supplements at or more than 1000 IU, but less than 2000 IU
- Normal TSH, PTH, and Total Calcium
- No family history of parathyroid or calcium regulatory disease. In addition, no personal history of kidney disease, GI disease, liver disease, and no bariatric surgery

The observed median, mean, and range between 2.5th to 97.5th percentile are summarized below

N	Mean	Median	2.5 th to 97.5 th Percentile
286*	29.7 ng/mL	27.7 ng/mL	12.7 – 65.7 ng/mL

* One sample <6.5 ng/mL was excluded from the data analysis

Each laboratory should establish its own reference range pertinent to their specific patient populations.

Instrument Name:

The BioPlex 2200 System, software version 4.1 cleared in k130053

Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.